

CLAIMS

1. A recombinant anticoagulant protein comprising a fusion of annexin V (ANV) (SEQ ID NO: 10) and a Kunitz protease inhibitor (KPI).
2. A recombinant anticoagulant protein according to Claim 1 comprising a protein sequence selected from the group consisting of TAP-ANV (SEQ ID NO: 1), ANV-6L15 (SEQ ID NO: 2), ANV-K_{APP} (SEQ ID NO: 3), and ANV-KK_{TFPI} (SEQ ID NO: 4), or conservatively substituted variants thereof.
3. An antithrombotic composition comprising a recombinant anticoagulant protein comprising a fusion of annexin V (ANV) (SEQ ID NO: 10) and a Kunitz protease inhibitor (KPI).
4. An antithrombotic composition according to Claim 3 further comprising a pharmaceutically acceptable excipient.
5. An antithrombotic composition according to Claim 3 comprising a protein sequence selected from the group consisting of TAP-ANV (SEQ ID NO: 1), ANV-6L15 (SEQ ID NO: 2), ANV-K_{APP} (SEQ ID NO: 3), and ANV-KK_{TFPI} (SEQ ID NO: 4), or conservatively substituted variants thereof.
6. A method of inhibiting blood coagulation in a mammalian subject comprising administering to the subject an effective amount of a recombinant anticoagulant protein comprising a fusion of annexin V (ANV) (SEQ ID NO: 10) and a Kunitz protease inhibitor (KPI).
7. A method of producing a recombinant anticoagulant protein comprising linking annexin V (ANV) (SEQ ID NO: 10) and a Kunitz protease inhibitor (KPI).
8. A method according to claim 7 comprising generating a recombinant DNA molecule comprising a first DNA sequence encoding annexin V (ANV) (SEQ ID NO: 9) and a second DNA sequence encoding a Kunitz protease inhibitor (KPI).
9. A method according to claim 8 comprising generating a recombinant DNA molecule comprising a sequence selected from the group consisting of TAP-ANV (SEQ ID NO: 5), ANV-6L15 (SEQ ID NO: 6), ANV-K_{APP} (SEQ ID NO: 7), and ANV-KK_{TFPI} (SEQ ID NO: 8), or conservatively substituted variants thereof.

10. A method of treating or preventing an excess of thrombotic activity in a subject in need of such treatment or prevention, said method comprising administering to the subject an effective amount of an antithrombotic composition comprising a fusion of annexin V (ANV) (SEQ. ID NO: 10) and a Kunitz protease inhibitor (KPI).
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11. A recombinant DNA molecule comprising a first DNA sequence encoding annexin V (ANV) (SEQ ID NO: 9) and second DNA sequence encoding a Kunitz protease inhibitor (KPI).
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12. A recombinant DNA molecule according to claim 11 wherein said first DNA sequence comprises SEQ. ID. NO. 9 (encoding ANV), SEQ. ID. NO. 14 (encoding Cys³¹⁵-to-Ala mutation of ANV), or conservatively substituted variants thereof.
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13. A recombinant DNA molecule according to claim 11 comprising a DNA sequence selected from the group consisting of TAP-ANV (SEQ ID NO: 5), ANV-6L15 (SEQ ID NO: 6), ANV-K_{APP} (SEQ ID NO: 7), and ANV-KK_{TFPI} (SEQ ID NO: 8), or conservatively substituted variants thereof.
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14. A host cell comprising a recombinant DNA molecule according to claim 11.
15. A stably transfected cell line expressing a recombinant anticoagulant protein according to claim 1.
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16. A prokaryotic cell line according to claim 15.
17. A eukaryotic cell line according to claim 15.
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18. A process for the preparation of a cell line expressing a recombinant anticoagulant protein comprising a fusion of annexin V (ANV) (SEQ ID NO: 10) and a Kunitz protease inhibitor (KPI), said process comprising stably transfecting a host cell with a recombinant expression vector comprising a cDNA sequence encoding ANV or conservatively substituted variants thereof, and a cDNA sequence encoding a KPI.
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19. A recombinant expression vector comprising a first nucleotide sequence encoding annexin V (ANV) (SEQ ID NO: 9), Cys³¹⁵-to-Ala mutation of ANV (SEQ ID NO: 14), or conservatively substituted variants thereof, and a second nucleotide sequence of a Kunitz protease inhibitor (KPI) together with additional sequences

capable of directing the synthesis of a recombinant anticoagulant protein comprising a fusion of ANV and a KPI, in a culture of stably transfected cells.

20. A recombinant expression vector according to claim 19 in a culture of stably transfected prokaryotic cells.
- 5 21. A recombinant expression vector according to claim 19 in a culture of stably transfected eukaryotic cells..
22. A recombinant expression vector according to claim 19 wherein said first nucleotide sequence comprises SEQ ID NO: 9 (encoding ANV), SEQ. ID. NO. 14 (encoding Cys³¹⁵-to-Ala mutation of ANV), or conservatively substituted variants thereof.
- 10 23. A recombinant expression vector according to claim 19 comprising a nucleotide sequence selected from the group of TAP-ANV (SEQ ID NO: 5), ANV-6L15 (SEQ ID NO: 6), ANV-K_{APP} (SEQ ID NO: 7), and ANV-KK_{TFPI} (SEQ ID NO: 8), or conservatively substituted variants thereof.

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